

12. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is separate from the DNA construct comprising the repressor gene, and wherein the constructs are introduced simultaneously into a plant cell
5 capable of being transformed and regenerated into a whole plant.

13. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is the same as the DNA construct comprising the repressor gene, said DNA construct
10 further comprising a DNA sequence that allows independent segregation of the inserted repressible lethal gene and the inserted repressor gene.

14. A method of making a genetically modified plant comprising:

15 (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

20 (ii) a DNA construct comprising a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

(iii) a DNA construct comprising a second repressible lethal gene, the activity of whose gene product is lethal to
25 plant cells; and,

(iv) a DNA construct comprising a second repressor gene whose gene product is capable of repressing said second lethal gene activity;

(b) regenerating from said plant cell a whole plant; and,
(c) selecting a plant wherein the inserted first
repressor gene segregates independently from the inserted first
repressible gene, and wherein the inserted second repressor
5 gene segregates independently from the inserted second
repressible gene.

15. The method of claim 14 wherein the DNA construct
comprising the first repressible lethal gene is separate from
the DNA construct comprising the first repressor gene; wherein
10 the DNA construct comprising the second repressible lethal gene
is separate from the DNA construct comprising the second
repressor gene; and wherein selection is accomplished by
crossing the plants generated in step (b).

16. The method of claim 14 wherein the inserted first
15 repressible lethal gene and the inserted first repressor gene
are located on opposite sister chromosomes of a chromosome
pair, and wherein the inserted second repressible lethal gene
and the inserted second repressor gene are located on opposite
sister chromosomes of a chromosome pair.

20 17. The method of claim 14 wherein the first repressible
lethal gene is within the same DNA construct as the first
repressor gene, and wherein the second repressible lethal gene
is within the same DNA construct as the second repressor gene;
and wherein independent segregation is effected by use of a
25 site-specific recombinase or a transposase.

18. The method of claim 14 wherein the first repressible
lethal gene is linked to the second repressor gene, and wherein
the second repressible lethal gene is linked to the first
repressor gene.

19. The method of claim 14 wherein the first or second or both repressible lethal gene is under control of a tissue-specific promoter.

20. The method of claim 14 wherein:

5 (a) the DNA construct comprising the second repressible lethal gene also comprises a first specific DNA operator sequence, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor 10 and the first specific DNA operator sequence results in repression of the second lethal gene; and,

(b) the DNA construct comprising the first repressible lethal gene also comprises a second specific DNA operator sequence, wherein the second repressor is a bacterial repressor 15 capable of binding to the second specific DNA operator sequence, and wherein the binding between the second bacterial repressor and the second specific DNA operator sequence results in repression of the first lethal gene.

21. The method of claim 14 wherein the first or second or 20 both repressor gene is under control of a tissue-specific promoter.

22. The method of claim 14 wherein the first, or first and second, repressor gene is under control of a constitutive promoter.

25 23. The method of claim 1 or 14 wherein the repressor comprises an antisense RNA or a sense gene capable of inhibiting expression of the repressible lethal gene.

24. The method of claim 1 or 14 wherein the repressor comprises a ribozyme capable of inhibiting expression of the repressible lethal gene.
25. The method of claim 14 wherein either or both
5 repressible lethal genes is selected from the group consisting of: oncogenes 1 and 2 of *Agrobacterium*, oncogenes 1 and 2 of *Pseudomonas*, oncogene 4 of *Agrobacterium*, a gene encoding a ribosome inactivating protein, a gene encoding diphtheria A chain toxin, and a gene encoding a ribonuclease.
- 10 26. The method of claim 10 or 25 wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.
27. The method of claim 10 or 25 wherein the ribonuclease is Barnase or T1 ribonuclease.
28. The method of claim 14 wherein the DNA construct
15 comprising a first repressible lethal gene, or the DNA construct comprising a second repressible lethal gene, or both, further comprises a conditionally lethal gene.
29. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is separate from
20 the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is separate from the DNA construct comprising the second repressor gene, and wherein the constructs are introduced simultaneously into a plant cell capable of being transformed
25 and regenerated into a whole plant.
30. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is

the same as the DNA construct comprising the second repressor gene, said DNA constructs further comprising a DNA sequence that allows independent segregation of the inserted first repressible lethal gene from the inserted first repressor gene,
5 and that allows independent segregation of the inserted second repressible lethal gene from the inserted second repressor gene.

31. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the
10 DNA construct comprising the second repressor gene; wherein the DNA construct comprising the second repressible lethal gene is the same as the DNA construct comprising the first repressor gene, said DNA constructs being contained within a single plant transformation vector that allows independent segregation of
15 the DNA constructs.

32. The method of claim 31 wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

33. The method of any one of claims 5, 19 and 21 wherein
20 the tissue specific promoter is a seed specific promoter.

34. The method of claim 33 wherein the seed specific promoter is the phaseolin promoter.

35. The method of claim 1 or 14 wherein the plant further comprises a novel trait.

25 36. The method of claim 35, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

37. The method of claim 35 or 36, wherein the gene encoding the novel trait is linked to a repressible lethal gene.

38. The method of any one of claims 35 to 37, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

39. A method of producing, for a hybrid cross, a male parent comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently from the inserted repressible gene;

(b) regenerating from said plant cell a whole plant;

(c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

25 wherein the homozygous plant can function as the male parent for a hybrid cross.

40. A method of maintaining the male parent of claim 39, comprising:

- (a) permitting the homozygous plant to self-pollinate;
- (b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and
- (c) obtaining the seed.

41. A plant produced by the method of claim 39 or 40

42. A method of producing, for a hybrid cross, a female parent that carries a repressible lethal gene, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

- (b) regenerating from said plant cell a whole plant.

43. A method of producing, for a hybrid cross, a female parent comprising a repressible lethal gene and an inducible repressor gene, comprising:

20 (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

- (i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently
5 from the inserted repressible gene;

(b) regenerating from said plant cell a whole plant;

(c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

10 wherein the homozygous plant can function as the female parent for a hybrid cross.

44. A method of maintaining the female parent of claim 43 comprising:

(a) permitting the homozygous plant to self-pollinate;

15 (b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and

(c) obtaining the seed.

45. A plant produced by the method of any one of claims
20 42 to 44.

46. The method of any one of claims 39, 40, 42 to 44 wherein said seed specific promoter comprises the phaseolin promoter.

47. The method of any one of claims 39, 40, 42 to 44
25 wherein the plant further comprises a novel trait.

48. The method of claim 47, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.
- 5 49. The method of claim 47 or 48, wherein the gene encoding the novel trait is linked to a repressible lethal gene.
50. The method of any one of claims 47 to 49, wherein the gene encoding the novel trait is under the control of a seed specific promoter.
- 10 51. A method of producing hybrid seed comprising a repressible lethal gene and a repressor gene, comprising:
- (a) allowing the plant of claim 45 to flower;
- 15 (b) fertilizing said plant with pollen which comprises a repressor gene whose gene product is capable of repressing said repressible lethal gene; and,
- (c) recovering hybrid seed which comprises a repressible lethal gene and a repressor gene.
52. A method of producing, for a hybrid cross, a female parent that carries a repressor gene, comprising:
- 20 (a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressor gene;
- (b) regenerating from said plant cell a whole plant;
- 25 (c) generating from said plant a plant which is homozygous for the repressor gene;

wherein the homozygous plant can function as the female parent for a hybrid cross.

53. The method of claim 52 wherein the female parent is incapable of self-pollination.

5 54. A plant produced by the method of claim 52 or 53.

55. A method of making a genetically modified plant seed comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

10 (a) crossing the male parent of claim 41 with the female parent of claim 54, wherein the repressor gene product of the female parent is capable of repressing the repressible lethal gene of the male parent;

15 (b) harvesting hybrid seed from the male parent, comprising the repressible lethal gene from the male parent and the repressor gene from the female parent.

56. The method of claim 55 wherein the plant seed is derived from a self-incompatible plant species.

57. A plant produced by the method of any one of claims 1 to 38, 51, 55 and 56.

20 58. The plant of any one of claims 41, 45, 54 and 57, further comprising a marker gene.

59. The plant of claim 57 or 58, which is a dicot (*Dicotyledoneae*).

60. The plant of claim 57 or 58, which is a monocot (Monocotyledoneae).

61. The plant of claim 57 or 58, which is a member of the family: *Malvaceae*, *Linaceae*, *Compositae*, *Fabaceae*, *Euphorbiaceae*, or *Oleaceae*.
62. The plant of claim 57 or 58, which is a member of the 5 genus: *Brassica*.
63. The plant of claim 57 or 58, which is a member of the genus: *Linum*, *Gossypium*, *Glycine*, *Arachis*, *Carthamus*, *Helianthus*, *Sinapis*, *Raphanus*, *Ricinus* or *Olea*.
64. The plant of claim 57 or 58, which is a member of the 10 family *Brassicaceae* (= *Cruciferae*).
65. The plant of claim 57 or 58, which is a *Brassica napus* or *rapa*.
66. The plant of claim 57 or 58, which is a rapeseed or canola.
- 15 67. The plant of claim 57 or 58, which is an oilseed plant.
68. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector, comprising:
- 20 (a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,
- (b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product; said repressible lethal gene and said repressor gene being located 25 so that they segregate independently.
69. A plant transformation vector, for use in limiting introgression of alien germplasm, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product; said
5 repressible lethal gene and said repressor gene being located so that they segregate independently.

70. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector or for limiting introgression of alien germplasm, comprising:

10 (a) a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

(b) a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

15 (c) a second repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(d) a second repressor gene whose gene product is capable of repressing said second lethal gene activity.

71. The vector of claim 70, wherein the first repressible lethal gene is not linked to the first repressor gene, and
20 wherein the second repressible lethal gene is not linked to the second repressor gene.

72. The vector of claim 70, wherein the first repressible lethal gene is linked to the first repressor gene, and wherein the second repressible lethal gene is linked to the second
25 repressor gene, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the first repressible lethal gene from the first repressor gene, and independent segregation of the second repressible

lethal gene from the second repressor gene, after transformation.

73. The vector of any one of claims 70 to 72, wherein:

(a) a first specific DNA operator sequence is linked to
5 the second repressible lethal gene, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor and the first specific DNA operator sequence results in repression of the second lethal
10 gene; and,

(b) a second specific DNA operator sequence is linked to the first repressible lethal gene, wherein the second repressor is a second bacterial repressor capable of binding to the second specific DNA operator sequence, and wherein the binding
15 between the second bacterial repressor and the second specific DNA operator sequence results in repression of the first lethal gene.

74. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising:

20 (a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(b) a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an
25 inducible promoter.

75. The vector of any one of claims 68, 69 and 74, wherein the repressible lethal gene and the repressor gene are not linked.

76. The vector of any one of claims 68, 69 and 74, wherein the repressible lethal gene and the repressor gene are linked, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the 5 repressible lethal gene and the repressor gene after transformation.

77. The vector of claim 72 or 76, wherein the at least one DNA sequence comprises a recognition sequence for a site-specific recombinase or a transposase.

10 78. The vector of any one of claims 68 to 77, wherein the repressible lethal gene or the repressor gene or both is under control of a tissue-specific promoter.

79. The vector of claim 78 wherein the tissue-specific promoter is a seed specific promoter.

15 80. The vector of claim 79 wherein the seed specific promoter is the phaseolin promoter.

81. The vector of any one of claims 68, 69 and 74 to 77, wherein a specific DNA operator sequence is linked to the repressible lethal gene, wherein the repressor is a bacterial 20 repressor capable of binding to the specific DNA operator sequence, and wherein the binding between the bacterial repressor and the specific DNA operator sequence results in repression of the lethal gene.

82. The vector of any one of claims 68 to 77, wherein the 25 repressor gene is under control of a constitutive promoter.

83. The vector of any one of claims 68 to 77, wherein the repressor gene is under control of an inducible promoter.

84. The vector of any one of claims 68 to 77, wherein the activity of the lethal gene product comprises over-expression or under-expression of a naturally occurring plant growth regulating substance.

5 85. The vector of any one of claims 68 to 77, wherein the repressible lethal gene is selected from the group consisting of: oncogenes 1 and 2 of *Agrobacterium*, oncogene 4 of *Agrobacterium*, gene encoding a ribosome inactivating protein, gene encoding diphtheria A chain toxin, and a gene encoding a
10 ribonuclease.

86. The vector of claim 85 wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.

87. The vector of claim 85 wherein the ribonuclease is Barnase or T1 ribonuclease.

15 88. The vector of any one of claims 68 to 77, further comprising a conditionally lethal gene linked to the repressible lethal gene.

89. The vector of any one of claims 68 to 77, wherein the repressor gene is under control of a constitutive promoter.

20 90. The vector of any one of claims 68 to 77, wherein the product of the repressor gene comprises an antisense RNA or a sense gene or a ribozyme capable of inhibiting expression of the repressible lethal gene.

91. The vector of any one of claims 68 to 77, further
25 comprising a gene encoding a novel trait.

92. The vector of claim 91, wherein the novel trait comprises an altered phenotype, a protein not found in the

native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

93. The vector of claim 91 or 92, wherein the gene encoding the novel trait is linked to the repressible lethal 5 gene.

94. The vector of any one of claims 91 to 93, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

95. A plant transformation vector, for use in generating 10 a parent plant for a hybrid cross, comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter.

96. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising a repressor gene.

15 97. Plasmid pG1, pG14, pHASTet1, pGG-14.